Institute of Paper Science and Technology

# FOREST BIOLOGY

# PROJECT ADVISORY COMMITTEE

DECEMBER 18, 1990

# INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY

Atlanta, Georgia

## FOREST BIOLOGY

## PROJECT ADVISORY COMMITTEE

December 18, 1990

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**FOREST BIOLOGY**

**PROJECT ADVISORY COMMITTEE MEETING**

**MEAD COATED BOARD TECHNICAL INSTITUTE**
**555 14TH STREET**
**CONFERENCE ROOM # 2**

**TENTATIVE AGENDA - DECEMBER 18, 1990**

| | | |
|---|---|---|
| *10:00 - 10:15 a.m.* | *Welcome & Introductions*<br>*Anti-Trust Statement* | *Dinus/Stanton* |
| *10:15 - 11:00 a.m.* | *Committee Orientation*<br>*Purpose*<br>*Guidelines*<br>*Review of RAC Meeting* | *Yeske* |
| *11:00 - 11:45 a.m.* | *Loblolly Pine*<br>*Initiation*<br>*Maturation*<br>*Seed Composition* | <br>*Webb*<br>*Webb*<br>*Nagmani* |
| *11:45 - 12:00 noon* | *Douglas-fir* | *Nagmani* |
| *12:00 - 12:30 p.m.* | **LUNCH** | **IPST** |
| *12:30 - 01:15 p.m.* | *Hardwoods*<br>*Shoot Cultures*<br>*Genetic Transformation*<br>*Somaclonal Variation* | <br>*Ozturk*<br>*Webb/Mathis*<br>*Mathis* |
| *01:15 - 01:30 p.m.* | *Student Projects* | *Dinus* |
| *01:30 - 02:15 p.m.* | *Forward Planning*<br>*Key Issues*<br>*New Directions*<br>*Terms of Office*<br>*Officiers*<br>*Alternates/Substitutes*<br>*External Specialists*<br>*Minutes*<br>*Future Meetings* | *All* |
| *02:15 - 02:30 p.m.* | *Summary & Conclusions* | *Stanton/Dinus* |
| *02:30 p.m.* | **ADJOURNMENT** | |

# FOREST BIOLOGY
# PROJECT ADVISORY COMMITTEE

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FOREST BIOLOGY

PROJECT ADVISORY COMMITTEE

Project 3223-00

# MASS CLONAL PROPAGATION OF IMPROVED CONIFERS

(Dinus, Nagmani, Webb)

December 18, 1990

DUES-FUNDED PROJECT SUMMARY FORM

FY 90-91

Project Title: Mass clonal propagation of improved conifers

Project Code: SFTWD

Division: CBSD

Project Number: 3223-00

Staff: Dinus, Nagmani, Webb

Current FY Budget: $500,000

Program Objective: Develop reliable cell and tissue culture systems for mass clonal propagation of genetically improved softwoods

Program Areas: Reduced Operating Costs, Capital Effectiveness, Fiber Availability, Quality, and Cost, End-Use Performance

Rationale: Major increases can be obtained in fiber production, quality, and uniformity via mass cloning of genetically improved trees. Reliable cell and tissue culture systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, increased pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening for and selecting useful variants from culture could also lower costs and accelerate the pace of conventional tree breeding. In addition, such systems could be used to investigate fiber formation under highly controlled conditions, thereby facilitating study of factors regulating fiber length, diameter, flexibility, and other properties. Improved

growth obtained via mass cloning will reduce raw material costs and increase returns on capital invested in land and equipment. Greater uniformity of clonal plantations can lower both woodlands and mill operating costs as well as enhance end-use performance and foster development of new, value-added products.

FY 90-91 Goals:

1) Raise frequencies for initiation of embryogenic cultures in loblolly pine and Douglas-fir; obtain additional cultures for accelerated work on embryo maturation.

2) Increase frequencies of embryo maturation and seedling conversion in Norway spruce model system; extend best treatments to Douglas-fir and loblolly pine.

3) Establish guideposts for manipulating somatic materials via documenting course of zygotic embryo development, maturation, and germination as well as early growth and development of zygotic seedlings.

4) Explore initiation of embryogenic cultures from explants of more mature plant materials.

Accomplishments to Date: Past research on cell and tissue culture systems has brought somatic embryogenesis, one method of mass cloning, closer to commercialization. Embryogenesis in Norway spruce, our model system, is now reproducible and straightforward. Embryogenic cultures can be obtained from immature and mature seeds and from tissues of newly germinated seedlings. Last efforts to

produce mature embryos showed that using glutamine in lieu of ammonium nitrate as well as higher concentrations of abscisic acid foster maturation. Numbers of mature embryos, averaged over all treatments, were 130 per g of culture tissue; the best treatment yielded 750 per g.

In recent months, work on spruce has been limited to producing materials for growth regulator assays and anatomical or biochemical comparisons of somatic and zygotic structures at various stages of development. This tack allowed us to consolidate progress and focus on a few critical topics as well as to devote more resources to our target species. In the course of this work, we merged several techniques into a more precise method for quantifying embryo numbers and stages. Samples of tissues, embryos, and media were collected at various times during maturation and forwarded to collaborators for growth regulator assays. In addition, structures at various stages were collected for evaluation of anatomical and biochemical characteristics. Results from these undertakings should lead to improved definitions of somatic embryo quality and better media for maturation and germination. Advances in research on spruce somatic embryogenesis in recent times have been rapid, especially in Canada. As a result, we are considering cessation of spruce work, except that dealing specifically with conversion to seedlings and comparison of zygotic and somatic materials.

To increase loblolly pine initiation frequencies, developing open-

and control-pollinated cones containing early stage (precotyledonary) embryos were secured from Union Camp and Westvaco. Care was taken to obtain cones from mother trees that yielded responsive explants in earlier years. Whole gametophytes were excised and placed on various media as described in our Initiation Strategy Document. Early observations indicated that roughly 10% of the explants showed some response. These initial responses appeared random with regard to treatment, but subsequent behavior, browning and loss or proliferation, varied considerably with media composition and growth regulator type/level. Yields remain uncertain, but at least 25 cultures now exhibit embryogenic phenotypes and 7 are growing at rates comparable to levels experienced in past years. Microscopic assays to confirm yields will be completed as quickly as sample sizes permit. Significant cone numbers have been maintained in cold storage, and are being used to retest best treatments from the first summer experiments and to examine a number of related options. Plans have also been made for "winter initiation", and arrangements are being made to secure the necessary cones from a Brazilian subsidiary of Westvaco.

Loblolly pine embryogenic cultures initiated in earlier years have been and are being used to improve maturation frequencies. Major factors remain carbohydrate source and concentration as well as abscisic acid levels. Two large factorial experiments were planned to confirm earlier findings and test several new options. Installation, however, was delayed by problems with contamination

and slower than expected culture growth. Sufficient tissue should be available for installation by mid-January. Experiment designs, however, are being adjusted to account for smaller amounts of tissue, new thinking about the role of ABA, and information from a recent patent.

Difficulties with initiation, proliferation, and maturation in our target species prompted reevaluation of past work on physical and chemical properties of zygotic embryos, gametophytes, and seeds. Results presented in early 1990 suggested that conventional media differ from environments afforded by developing seed. Efforts were therefore undertaken to reassess mineral composition, cations and anions, in developing seed, with multiple collections made from just after fertilization to the fully ripened state. Open-pollinated cones from six seed orchard mother trees were provided by Weyerhaeuser. Analyses of material from most collections have been completed, but fully ripened seeds have not yet been secured, processed, or analyzed.

Initiation in Douglas-fir was deferred in order to concentrate resources on loblolly pine. Exploratory trials on embryo maturation, however, showed much promise. More definitive tests with three tissue sources were planned and are in progress.

Related Projects: The softwood project is complemented by collaboration with scientists in several external organizations.

expertise are exchanged freely. Some examples are:

Dr. D. Gray, Univ. of FL, Leesburg - Readying Norway spruce somatic embryos for storage and germination via desiccation.

Drs. L. Barbour, SAPPI and J. Cutting, Stellenbosch Univ. Quantifying ABA in embryogenic and nonembryogenic cultures and developing zygotic and somatic embryos.

Dr. K. Eriksson, Univ. of GA - Lignin precursors as indicators of maturation in developing embryos and seedlings. This should also contribute to eventual use of our cultures as vehicles for studying lignin biosynthesis and deposition.

Dr. D. Neale, US Forest Service - Expression of foreign genes in cultures of commercially important softwoods (pending).

Related Student Projects:

Completed in 1990

Michael Wood - M.S., Effect of cold shocking on cell cultures of Larix decidua. Advisor, Dinus.

In Progress

David Barzyk - M.Sc., Development of a fiber optics system to determine the in vivo pH of developing Pine taeda seeds. Advisor, Dinus.

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Project 3223-02

BIOCHEMISTRY OF CLONAL PROPAGATION

(Dinus, Mathis)

December 18, 1990

DUES-FUNDED PROJECT SUMMARY FORM

FY 90-91

Project Title: Biochemistry of Clonal Propagation

Project Code: BIOCM

Division: CBSD

Project Number: 3223-02

Staff: Dinus, Mathis

Proposed FY 90-91 Budget: $150,000

Project Objective: Develop an understanding of biochemical mechanisms controlling embryogenesis and other cloning methods, and devise procedures for raising effectiveness and efficiency of mass cloning methods

Program Areas: Reduced Operating Costs, Capital Effectiveness, Fiber Availability, Quality, and Cost, Environmental Impact, End Use Performance

Rationale: Improved understanding of biochemical mechanisms underlying successful cloning processes will shorten time to commercial application of clonal forestry, raise its efficiency, and facilitate extension to trees old enough to have been proven genetically superior. Understanding the biochemistry of cell and tissue cultures will also facilitate their application to other problems; e.g., fiber formation and lignification.

FY 90-91 Goals:

1) Complete restaffing and equipping of laboratory.

2) Renew work on biochemical similarities/differences of developing

somatic and zygotic embryos, with emphasis on storage proteins and lipids.

3) Develop techniques for obtaining herbicide tolerance in selected hardwood species via genetic transformation and/or somaclonal variation/selection.

4) Adapt molecular techniques for verifying genetic fidelity and gene expression.

Accomplishments to Date: Past efforts have made somatic embryogenesis in Norway spruce, our model system, straightforward and reproducible. Embryos can be produced in large numbers, and somatic seedlings have been recovered. Somatic embryogenesis has also been obtained in our target species, loblolly pine and Douglas-fir, but initiation and maturation frequencies remain low and seedlings have not been recovered.

Earlier work on the biochemistry of embryogenesis yielded useful data on differences between embryogenic and nonembryogenic cultures, and some knowledge of factors affecting the process. Such differences and associated markers can be used to screen cultures for embryogenic potential, and monitor effects of modified or new protocols. In addition, techniques for isolating, purifying, and characterizing proteins, lipids, enzymes, RNA, and DNA have been developed or adapted for use with forest trees. These are now available for application toward increasing initiation and maturation frequencies, facilitating conversion to

seedlings, and evaluating seedling performance and fidelity.

More recently, recruiting and hiring efforts resulted in addition of a molecular biologist and two technical staff personnel. The biochemistry/molecular biology laboratory has been organized and is being used by new employees as well as other staff and students. Efforts to acquire new equipment and supplies are underway.

Actions needed to secure the laboratories for recombinant DNA research were completed, and we have been certified as in compliance with all applicable USDA and NIH regulations. Permits for acquisition, storage, and handling of Agrobacterium tumefaciens and other such materials were secured ahead of schedule. A comprehensive chemical inventory system has also been developed and implemented.

Plans for obtaining herbicide tolerance in hardwoods were developed and are being implemented. Efforts are focused on gene transfer, with somaclonal variation/selection as insurance. A working dialogue was established with technical and legal representatives of Monsanto Corp. in connection with their providing the Institute with genes for research and/or commercialization. A genetic construct for enhanced auxin synthesis has been received for use in student research. Negotiations concerning a gene for glyphosate tolerance are continuing. Additional information on these topics is provided on the Project Summary Form for Project 3223-03.

Experiments on mechanisms underlying beneficial effects of maltose on loblolly pine embryo maturation are being planned and should be implemented in the near future. Work on proteins and lipids in maturing loblolly and Douglas-fir embryos as well as on genetic fidelity of somatic embryos and seedlings is also being planned.

Related Projects: This project effort supports work underway in Projects 3223-00 and 3223-03.

Related Student Projects:

In Progress

James Bond - Ph.D., A Raman microspectroscopic investigation of the patterns of molecular order in secondary walls of southern pine tracheids. Committee participation, Dinus.

Colleen Walker - Ph.D., Development of a biomimetic approach for pulp bleaching. Advisor, Dinus.

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Project 3223-03

# MASS CLONAL PROPAGATION OF GENETICALLY IMPROVED AND ENGINEERED HARDWOODS

(Dinus, Mathis, Webb)

December 18, 1990

DUES-FUNDED PROJECT SUMMARY FORM

FY 90-91

Project Title: Mass clonal Propagation of Genetically Improved and Engineered Hardwoods

Project Code: HRDWD

Division: CBSD

Project Number: 3223-03

Staff: Dinus, Mathis, Webb

FY 90-91 Budget: $125,000

Project Objective: Develop reliable cell and tissue culture systems for mass clonal propagation of genetically improved and/or engineered hardwoods

Program Areas: Reduced Operating Costs, Capital Effectiveness, Fiber Availability, Quality, and Cost, End Use Performance

Rationale: Major increases can be obtained in fiber production, quality, and uniformity via mass cloning. Reliable cloning systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, greater pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening/selection for useful variants in tissue culture holds promise for raising the pace and efficiency of conventional tree breeding. In addition, such systems could be used to investigate fiber formation under highly controlled conditions, thereby facilitating study of factors governing fiber properties of interest to the industry. Accelerated growth will ensure reliable raw material supplies,

reduce their costs, and raise returns on capital invested in land and equipment. Greater uniformity can lower both woodlands and mill operating costs as well as enhance properties related to end-use performance. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

FY 90-91 Goals:

1) Complete construction and equipping of greenhouse.

2) Secure additional plant materials and establish "clean" greenhouse populations.

3) Expand existing cultures, and initiate or obtain and stabilize additional ones.

4) Refine technologies for mass propagation; ensure suitability for genetic transformation and/or somaclonal variation/selection.

5) Accelerate research on gene transfer and expression.

Accomplishments to Date: Considerable hardwood research has been done at the Institute in past years. This work resulted in production of plants from tissue culture, and successful application of polyploidy to forest tree breeding. Other exploratory work at the Institute suggested that tissue culture methods can be used to test for disease resistance. Results from these efforts and those of other organizations indicate that hardwood tissues, cells, and protoplasts can be manipulated with relative ease. In addition, the first demonstration of gene transfer and expression in forest trees was accomplished with a

hardwood. Still other work infers that novel variants can be produced in culture, isolated, and used to introduce new traits into breeding and/or planting stock.

In accordance with earlier plans, this project seeks to develop technologies for transferring genes for herbicide tolerance into commercially important species, and for efficient mass propagation, testing, and release of genetically modified plant materials. Herbicide tolerance is also sought, as a matter of insurance, via somaclonal variation and selection.

Greenhouse populations have been increased to include a total of seven cottonwood clones, five of which are widely used in breeding and planting programs. Twelve control-pollinated seedlots have been secured from James River Corp. to further expand numbers of improved genotypes available for research.

Shoot cultures of five additional cottonwood genotypes were established. The task proved difficult, especially for improved clones of southern origin. Numerous media and growth regulator combinations, along with ethylene inhibitors and anti-auxins, were tried before reasonable numbers of cultures were secured. To improve efficiency, an alternate protocol has been obtained from cooperators at Oregon State University and the University of Nebraska. This approach, scheduled for testing in the near future, involves brief exposure of stem internode explants to a callus-

forming medium followed by transfer to shoot induction medium. Six clones, including C175, are now being maintained for research. These, and new ones established in future, will be used to "generalize" the Leaf Section System, developed earlier this year, for transformation and propagation. Representative numbers of aspen and sweetgum cultures are also being maintained.

Efforts to "generalize" the Leaf Section System to more genotypes have slowed, primarily due to departure of our Post Doctoral Fellow. Results to date, however, appear promising, and work continues. In addition, student research has shown that the large numbers of explants needed for genetic transformation can be produced with ease.

As noted in the Project Summary Form for Project 3223-02, our facilities have been certified for work on genetic transformation and a working relationship has been established with Monsanto Corp. The auxin synthesis gene needed for student research has been obtained, and transformation of Clone C175 will be attempted in the near future. First tests of C175 sensitivities to antibiotics have been completed, a step required to ensure that transformed materials can be selected and that Agrobacterium tumefaciens can be removed following transformation. Efforts to finalize legal and technical arrangements for securing the Monsanto gene for glyphosate tolerance are ongoing, and should be expedited by progress with the auxin synthesis gene.

Suspension cultures, established earlier, are being maintained with relative ease. Cultures of C175 were used to develop growth curves; results are being used to better determine when to withdraw samples for other research and how frequently to subculture. Improved efficiencies in both research and routine maintenance should follow. Efforts to regenerate plants from C175 suspensions were successful. Significant numbers of plants were obtained by transferring microcalli, whose formation was reported earlier, to shoot induction and rooting media used for the Leaf Section System. Successful regeneration from suspensions, if workable for a variety of genotypes, will provide another avenue for transformation. Suspension cultures of several other genotypes have been established from stem internode explants, and are now being expanded for use in various lines of research. A detailed plan for obtaining herbicide tolerant plants via somaclonal variation/selection was developed, and experimentation has been started.

Related Projects: Contacts developed during work on a DOE subcontract are used as sources of useful plant material and cultures, methods for establishing and maintaining cultures, and expertise for genetic transformation. Cooperating organizations include: Oregon State University, University of Iowa, University of Kentucky, University of Nebraska, Tuskegee University, and the US Forest Service, Rhinelander, WI.

Related Student Projects

In Progress

Lois Forde - M.Sc., Phenylalanine ammonia lyase and lignin biosynthesis. Advisors, Conners and Dinus.

Jim Kramer - M.Sc., Pulping and papermaking properties of Florida-grown Eucalyptus amplifolia. Advisors, Dinus and McDonough.

Tom Ptacek - M.Sc., Variability of wood, fiber, and pulping properties as affected by cloning. Advisor, Dinus.

Peasely Shorter - M.Sc., Promotion of additional auxin synthesis in Populus deltoides via transformation with Agrobacterium tumefaciens. Advisor, Webb.

# Initiation of Embryogenic Callus from Megagametophytes of *Pinus taeda*

D.T. Webb, N. Rangaswamy - Principal Investigators

D. Evans, Y. Powell, C. Stephens - Technical Assistants

# OBJECTIVES

1. Increase frequency of initiation

2. Insight regarding underlying factors

3. New lines for maturation & development

# EXPERIMENTAL APPROACH

1] Donor (Mother) Trees

2] Embryo Stage

3] Whole Megagametophyte

4] Growth Regulators

5] Culture Media

6] Amino Acids & Amides

## RESULTS - 1 - EXTRUDED CALLUS

A. Collection 1 cones -> Stage 2-3 (1:1) Zygotic Embryos

B. Collection 2 cones -> Stage 3-4 (1:1) Zygotic Embryos

C. 90% of megagametophytes contained Zygotic Embryos

D. All Source Trees & Collections -> Extruded Callus

E. Overall Extrusion Frequency (DCR & MSG) = 7.6%

(Corrected for empty megagametophytes = 8.4%)

1. MSG = 8.0% (Fig. 1; Corrected = 8.8%)

2. DCR = 7.25% (Fig. 2; Corrected = 8.0%)

F. Extrusion Frequency BM = 2.2%

# RESULTS - 1 - EXTRUDED CALLUS

A. Best Treatments (Corrected Frequencies)

1. DCR #2 (1.1 mg/l 2,4-D) = 14.6%
2. DCR #6 (2 mg/l 2,4-D + 1.0 mg/l BA) = 15.1%
3. MSG #2 (1.1 mg/l 2,4-D) = 13.5%
4. MSG #4 (2 mg/l 2,4-D) = 12.5%
5. MSG #11 (5 mg/l 2,4-D + 0.5 mg/l BA) = 13.3%
6. MSG #12 (5 mg/l 2,4-D + 1 mg/l BA) = 13.7

B. Best Common Treatment -> #2 (1.1 mg/l 2,4-D)

1. MSG = 12.25% (Corrected = 13.5%)
2. DCR = 13.3% (Corrected = 14.6%)

# RESULTS - 1 - EXTRUDED CALLUS

## A. Best Cumulative Treatments (Fig.3)

1. #2 (1.1 mg/l 2,4-D)
2. #9 (3.0 mg/l 2,4-D + 1.0 mg/l BA)
3. #10 (5.0 mg/l 2,4-D)
4. #11 (5.0 mg/l 2,4-D + 0.5 mg/l BA)

## B. Worst Treatment -> #1 (1.1 mg/l 2,4-D)

1. MSG = 4.5% (Corrected = 4.9%)
2. DCR = 4.25% (Corrected = 4.7%)

## RESULTS - 2 - INDEPENDENT CALLUS

### EXPLANT SOURCE

A. All Source Trees -> Independent Callus (Table 1)

B. Both Collections -> Independent Callus

C. Excised Embryos did not proliferate

# RESULTS - 2 - INDEPENDENT CALLUS

## BASAL MEDIUM

A. All Basal Media -> Independent Callus (IC)

B. DCR (16 Lines[1]) > MSG (7 Lines[1]) > BM (1 Line)

C. Only DCR -> Proliferating Callus (P; 15 Lines)

[1] One line (#7; Table 1) initiated on G0 medium -> D1 medium

## RESULTS - 2 - INDEPENDENT CALLUS

### DCR MEDIUM

A. 69% IC Initiated & Maintained on 2,4-D alone

B. 50% of Rapidly Proliferating (P*) Calli

   Initiated & Maintained on 2,4-D alone

C. 83% P* calli maintained on 2,4-D alone

### MSG & BM MEDIA

A. MSG 14% IC initiated on 2,4-D alone

B. BM -> only one IC

## EXPERIMENT IN PROGRESS

1. Union Camp cones - one source tree
2. Stage 3 vs 4 zygotic embryos (2 collections)
3. Megagametophyte Culture
4. DCR Medium

   D1 = 1.1 mg/l 2,4-D (Best medium from work above)

   D8 = 3 mg/l 2,4-D + 0.5 mg/l BA (Old Maintenance Medium)
5. Full-Strength vs Half-Strength salts
6. Sucrose 3% vs Maltose (equimolar)

## SUMMARY & CONCLUSIONS

1. Whole megagametophyte culture equals or surpasses embryo culture for initiation frequency [ie. extruded callus].
2. Megagametophyte cultures are far easier to inoculate & transfer than excised embryos.
3. Uncallused & Undamaged megagametophytes -> extruded callus. Thus, origin of EC is unambiguous. However, exceptions occur and in some cases origin could be uncertain.
4. Extruded callus probably represents secondary embryos, as dominant embryo is often at chalazal end of megagametophyte.

## SUMMARY & CONCLUSIONS

5. Extrusion occurs on all media & does not require growth regulators.
6. Levels of Glutamine, Asparagine & Arginine based on 7/6 analysis may be better than 6/30 analysis for initiation. However, no proliferating callus developed.
7. Tetrazolium tests show that culture medium is rapidly delivered to the embryo and inner megagametophyte. Thus, extrusion is not limited by any diffusion barrier.
8. DCR & MSG media -> similar overall results for extrusion.
9. BM medium was inferior
10. There is no consistent pattern for extrusion on different media treatments.

## SUMMARY & CONCLUSIONS

11. No agreement between media which favor extrusion and those which support proliferation.

    Worst Initiation Medium = Best Proliferation Medium

12. In most cases, embryo heads turn brown and extruded callus declines. This suggests an oxidation problem.
13. DCR is better than other media for callus proliferation.
14. Several DCR media are suitable for proliferation
15. Use of 2,4-D without cytokinin may be better for initiation & maintenance.

# NEXT STEPS/OPTIONS

1. Source Trees & Explants
   - A. Cones from Brazil
     1. Five Clones
     2. Three Collections -> Stages 2-4 (precotyledonary)
   - B. Union Camp Cones
     1. One Clone -> Megagametophyte Culture (In Progress)
     2. Two Clones -> Embryo Culture
        - a. Time & Resources
        - b. Viability after storage
2. Explant
   - A. Whole Megagametophyte
   - B. Half Megagametophyte (Transverse Bisect)
   - C. Punctured
     1. Chalaza -> Dominant Embryo
     2. Micropyle -> Higher Extrusion Frequency
   - D. Remove Embryogenic Material at Micropyle [suction]

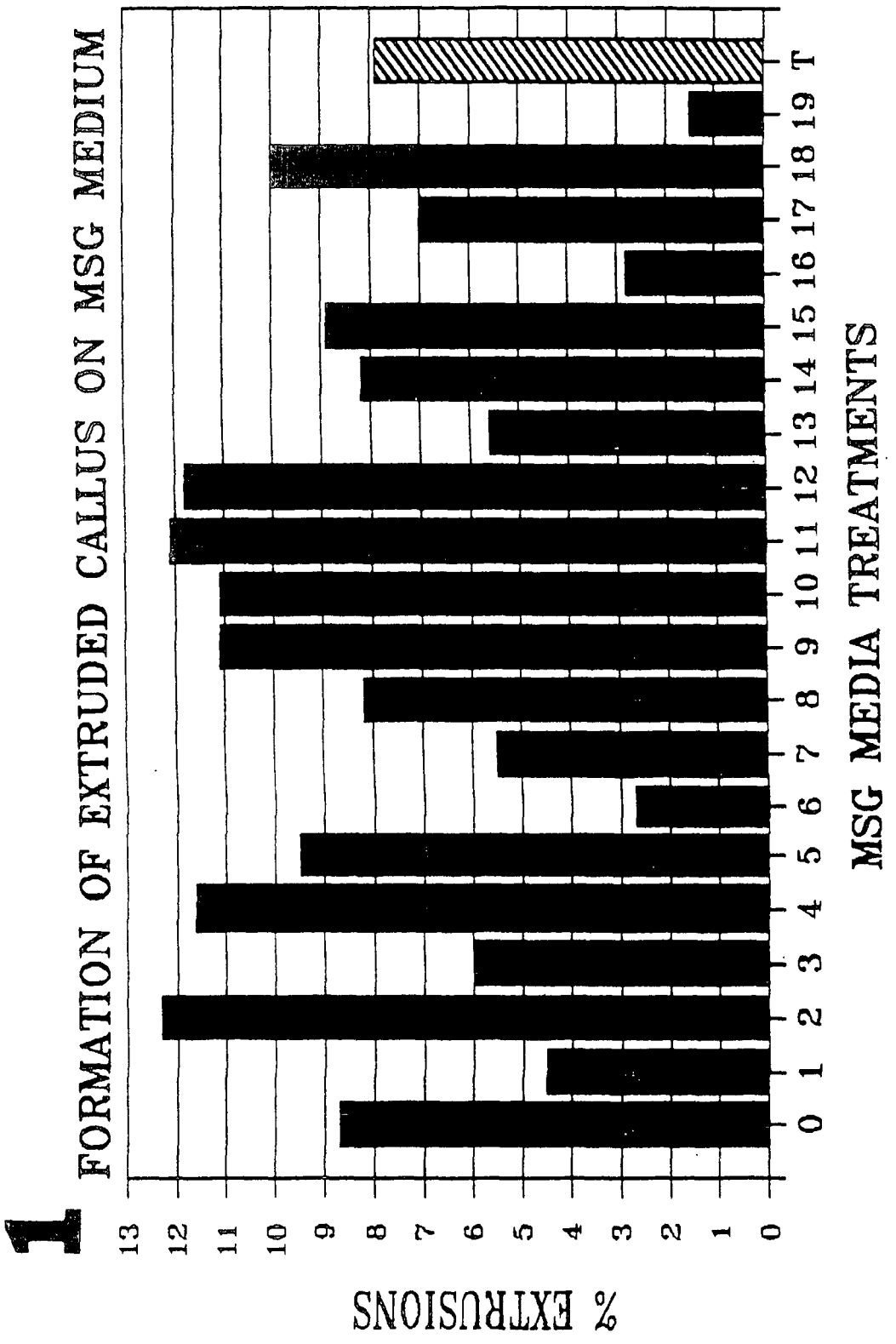
# NEXT STEPS/OPTIONS

3. Growth Regulators
   - A. 2,4-D alone (1.1, 3 mg/l)
   - B. 3 mg/l 2,4-D + 0.5 mg/l BA
4. Basal Medium = DCR
   - A. Full-Strength Salts & Nitrogen Sources
   - B. Half-Strength Salts & Nitrogen Sources
   - C. pH Buffer
   - D. Anti-oxidants (See 7 below)
5. Carbohydrates
   - A. Maltose vs Sucrose (Various Concentrations)
   - B. Glucose vs Sucrose

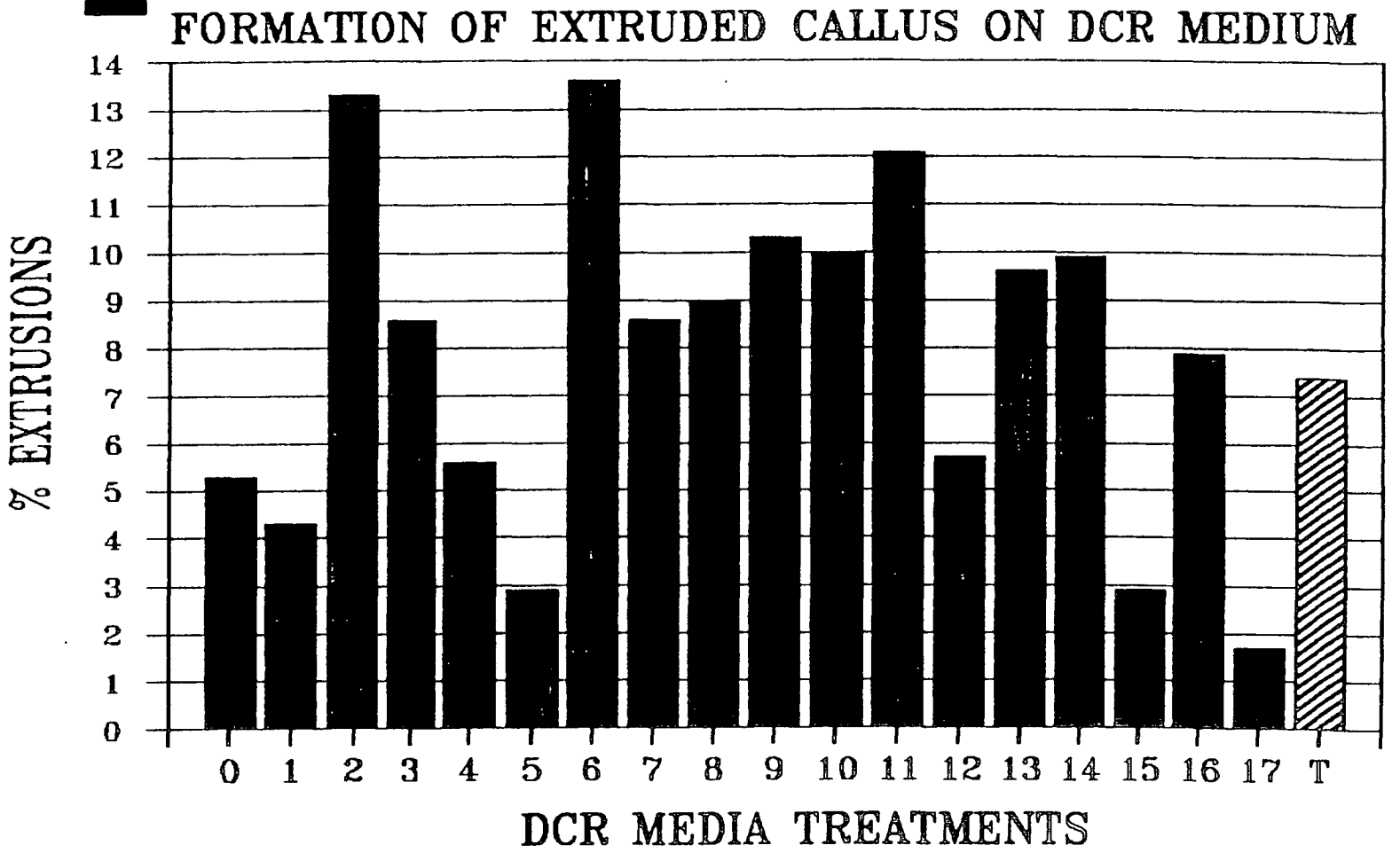
## NEXT STEPS/OPTIONS

6. Gelling Agent & Support
   - A. Washed Agar
   - B. Bactoagar
   - C. Gelrite
   - D. Gellan Gum
   - E. Liquid with inert support
     1. (Sorborods = Cellulose)
     2. Membrane or Screen
7. Culture Environment
   - A. Reduced Oxygen Levels (Carmen, 1988; "Oxyreducer")
   - B. Liquid Medium

     (Need novel approach ?co-cultivation wells?)
   - C. Others ????
8. Nitrogen Sources = Too complicated - pursue later
9. OTHERS ???


1 FORMATION OF EXTRUDED CALLUS ON MSG MEDIUM
% EXTRUSIONS
0 1 2 3 4 5 6 7 8 9 10 11 12 13
MSG MEDIA TREATMENTS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 T

**2**


FORMATION OF EXTRUDED CALLUS ON DCR MEDIUM
% EXTRUSIONS
0
1
2
3
4
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6
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DCR MEDIA TREATMENTS

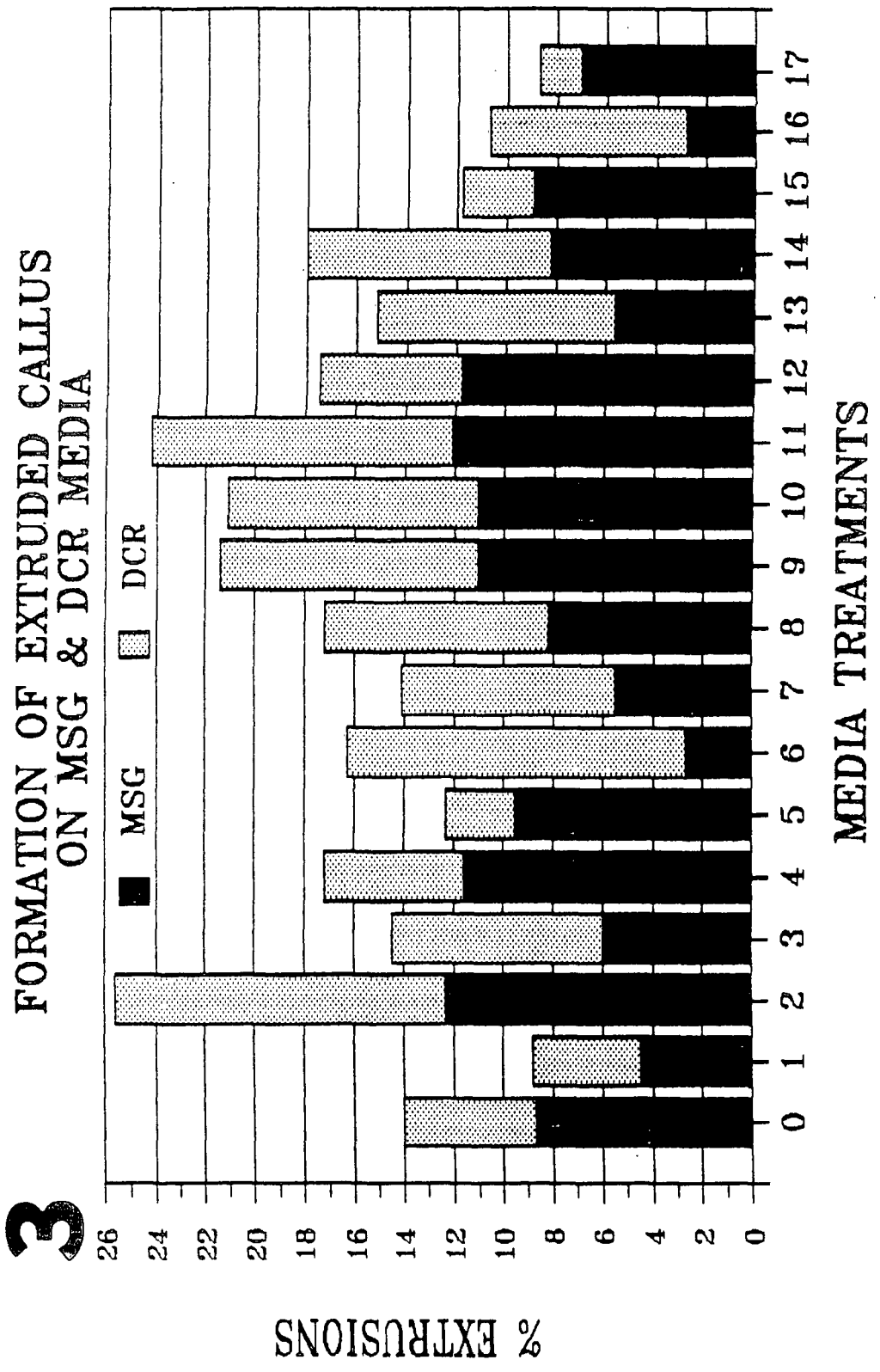

3
FORMATION OF EXTRUDED CALLUS
ON MSG & DCR MEDIA
MSG
DCR
% EXTRUSIONS
0
2
4
6
8
10
12
14
16
18
20
22
24
26
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
MEDIA TREATMENTS

Table 1. SUMMARY OF INITIATION WITH WESTVACO CONES

| LINE ID | SOURCE | INITIATION MEDIUM | MAINTENANCE MEDIUM | STATUS |
|---|---|---|---|---|
| **DCR MEDIUM** | | | | |
| 1] | W1-1-1 | D1 [B3] | D1 | P[1] |
| 2] | W2-1-1 | D1 [B2] | D1 | S[2] |
| 3] | W2-1-2 | D1 [A2] | D1 | P[3] |
| 4] | W3-2-1 | D4 [A1] | D1 | P |
| 5] | W3-1-1 | D1 [B2] | D1 | P[#4] |
| 6] | W3-1-1 | D8 [C4] | D1 | P[#] |
| 7] | W3-1-1 | D9 [B4] | D1 | P |
| 8] | W3-1-1 | D11 [B2] | D1 | P |
| 9] | W1-1-2 | D12 [B2] | D1 | P |
| 10] | W3-1-1 | G0 [A2] | D1 | P[#] |
| 11] | W3-1-2 | D1 [C1] | D1 | P |
| 12] | W3-1-2 | D7 [B1] | D1 | P |
| 13] | W1-1-1 | D3 [B3] | D3 | ?? |
| 14] | W2-1-2 | D4 [A2] | D4 | P* |
| 15] | W1-1-1 | D5 [A1] | D5 | P* |
| 16] | W1-1-1 | D7 [C3] | D7 | P |
| 17] | W1-1-1 | D7 [C4] | D7 | P |
| 18] | W3-1-2 | D7 [B1] | D7 | P* |
| 19] | W1-1-1 | D10 [C4] | D10 | P |
| 20] | W3-1-2 | D17 | D17 | P[1] |

**SUMMARY 20 LINES**

**2 SOLITARY**

**17 PROLIFERATING**

**6 PROLIFERATING PROFUSELY**

MSG MEDIUM

| | | | | |
|---|---|---|---|---|
| 21] | W1-1-2 | G0 [C4] | G6 | s |
| 22] | W3-2-1 | G1 [A4] | G6 | s |
| 23] | W3-2-1 | G2 [A1] | G6 | s |
| 24] | W3-2-1 | G2 [B4] | G6 | s |
| 25] | W2-1-1 | G14 [A2] | G14 | s |
| 26] | W3-2-1 | G15 [A1] | G15 | s |
| 27] | W2-1-1 | G16 [C3] | G16 | s |

---

SUMMARY 7 LINES

ALL SOLITARY

---

BM MEDIUM

| | | | | |
|---|---|---|---|---|
| 28] | W2-1-2 | BM11 [B2] | BM1 | s |

---

SUMMARY 1 LINE

SOLITARY

---

[1] Two - four callus clumps

[2] Solitary callus

[3] Four - eight callus clumps

[4] More than eight callus clumps

# Role of Carbohydrates and Growth Regulators in the Maturation and Development of *Pinus taeda* Somatic Embryos

D.Webb, J.Mathis - Principal Investigators

S. Ozturk, Y. Powell - Technical Assistants

# OBJECTIVES

1. Confirm results which show that maltose is better than sucrose for the maturation of precotyledonary & cotyledonary somatic embryos

2. Certify that glucose is superior to sucrose and inferior to maltose

3. Compare the effects of filter-sterilized vs autoclaved carbohydrates

4. Determine whether the beneficial effects of maltose & glucose are primarily nutritional or osmotic

5. Evaluate whether or not ABA is required

6. Establish the optimal interaction of ABA with carbohydrates

7. Determine the interaction of auxin with ABA & carbohydrates

8. Understand the key molecular events evoked by carbohydrates

## SUMMARY & CONCLUSIONS OF PRIOR RESULTS

1. Lob pine callus does not -> cotyledonary embryos with Sucrose

2. Maltose and Glucose -> cotyledonary embryos

3. Maltose was superior to Glucose

4. Effective Maltose levels range from 0.17 to .7M

5. Results are fragmentary and optimal levels are unknown

6. All carbohydrates were autoclaved

7. ABA (20-30 uM) is required for somatic embryo development

8. Optimal ABA carbohydrate interaction levels are unknown

9. IBA may enhance cotyledonary embryo production

10. Changes in the sequence of carbohydrate & hormone treatments may yield superior results

# EXPERIMENTAL APPROACH

1. Determine optimal levels for Maltose, Glucose vs Sucrose with 30 uM ABA
    - A. Autoclaved with media
    - B. Filter-sterilized
2. Test the interaction of optimal carbohydrate levels with ABA doses
3. Determine the osmotic component of the carbohydrate effect
    - A. Non-physiological osmotica
        1. Sorbitol
        2. Mannitol
    - B. Physiological osmotica
        1. Inositol
4. Test the interaction of IBA with the above
5. Determine the best sequence of treatments
6. Explore the effects of suspension culture vs callus
7. Determine the effects of key treatments on gene expression

# NEXT STEPS/OPTIONS

1. **Compare effects of Maltose & Glucose vs Sucrose with callus**
    - A. Two best lines
    - B. Additional two lines
    - C. New Lines
2. **Preliminary studies on osmotic effects**
3. **Interaction of ABA & Carbohydrates**

# ELEMENTAL COMPOSITION OF DEVELOPING OVULES OF LOBLOLLY PINE ..... A MECHANISTIC APPROACH

NAGMANI, R. RON HOOPER JACINTA CASTELLINO RON DINUS

## OBJECTIVES

REVIEW PAST I.P.S.T RESEARCH

COMPLETE DATA ON OVULE COMPOSITION

COMPARE WITH EXISTING SYNTHETIC CULTURE MEDIA

RECOMMEND PROTOCOL CHANGES IF POSSIBLE

## METHODS

ICP - EMISSION SPECTROMETRY

ION CHROMATOGRAPHY

TABLE 1 : GENOTYPES, COLLECTION DATES & DEVELOPMENTAL STAGE OF ZYGOTIC EMBRYO IN LOBLOLLY PINE (SEED CONE COLLECTION FROM LYONS, GA ( WEYERHAUSER CO )

| GENOTYPE ( MOTHER TREES ) | DATE OF SEED CONE COLLECTION | STAGE OF OVULE/ EMBRYO DEVELOPMENT |
|---|---|---|
| 1A; 2B; 3C<br><br>4D;P1 & P2<br><br>(6 MOTHER TREES) | 7/2/90<br><br>7/9/90<br><br>7/16/90<br><br>7/23/90<br><br>8/1/90 ---- 8/31/90 (WEEKLY INTERVALS) | POST-FERTILIZATION STAGES BEGINNING PRE-COTYLEDENORY & COTYLEDONARY<br><br>( SEE FIGS) |

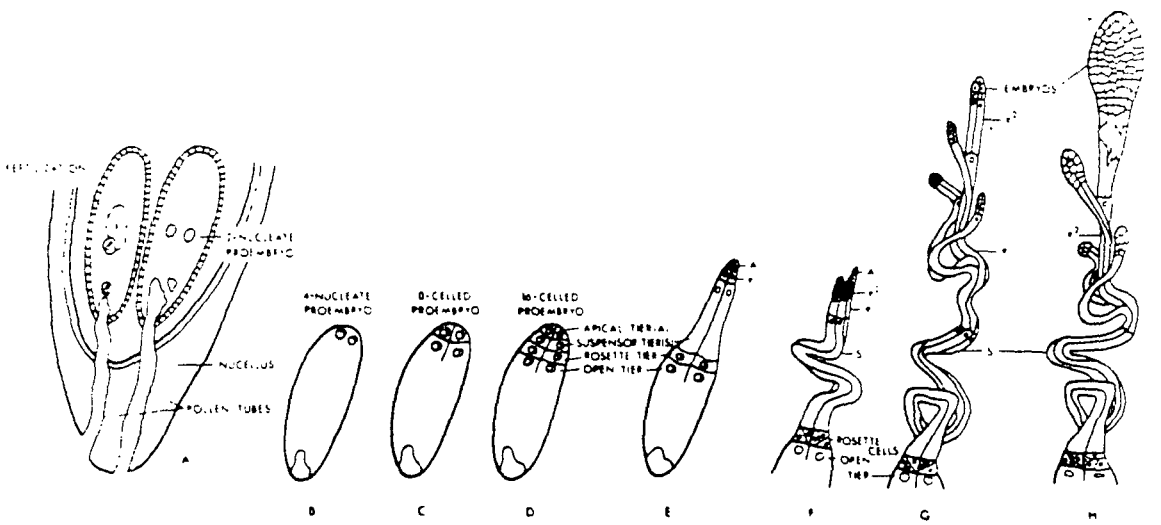



Figure 7.2 Fertilization and cleavage polyembryony in Pinus (from Owens and Molder 1984b).

## RESULTS

TABLE 2: ELEMENTAL ANALYSIS OF LOBLOLLY PINE OVULES
( CATION ANALYSIS )

| ug/gram of oven dried samples; Dry.wt. | | |
|---|---|---|
| MACROELEMENTS | 7/2/90 * | 7/16/90 * |
| CALCIUM | 260 | 320-350 ** |
| MAGNESIUM | 11,900 | 9,300-9,600** |
| PHOSPHORUS | 17,300 | 18,200-21,000** |
| POTASSIUM | 36,900 | 15,200-15,400** |
| SODIUM | 13.2 | 6.21-7.77** |

* COLLECTION DATES OF SEED CONES FROM LYONS, GA

** DATA FROM DUPLICATE SAMPLES

## RESULTS

TABLE 2: ELEMENTAL ANALYSIS OF LOBLOLLY PINE OVULES
( CATION ANALYSIS )

| MICRO-ELEMENTS | ug/gram of oven dried samples; Dry. wt. 7/2/90* | 7/16/90* |
|---|---|---|
| BORON | 70 | 43-48** |
| MANGANESE | 280 | 420-430** |
| ZINC | 237 | 210-240** |
| MOLYBDENUM | <0.125 | < 0.125 |
| COPPER | 19 | 27-26 ** |
| COBALT | <0.075 | < 0.075 |
| NICKEL | 8.4 | 6.88-7.28** |
| IRON | 110 | 92-99** |

* COLLECTION DATES OF SEED CONES FROM LYONS, GA

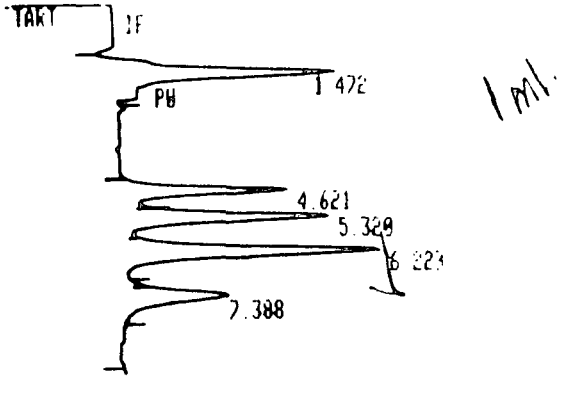
** DATA FROM DUPLICATE SAMPLES

RUN # 55 SEP/28/90 12:49:04

AREA%

| RT | AREA | TYPE | AR/HT | AREA% |
|---|---|---|---|---|
| 1.475 | 1.0126E+07 | D BB | 0.362 | 29.698 |
| 4.634 | 4173700 | PB | 0.226 | 12.241 |
| 5.328 | 6319000 | BB | 0.270 | 18.533 |
| 6.232 | 9320400 | BB | 0.303 | 27.336 |
| 7.401 | 4156800 | BB | 0.359 | 12.192 |

TOTAL AREA= 3.4096E+07
MUL FACTOR= 1.0000E+00



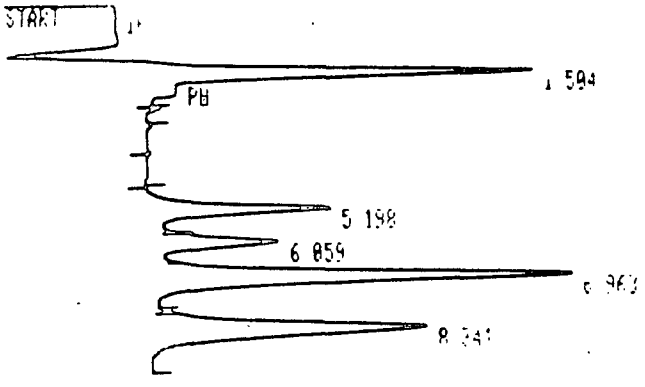

STOP

RUN # 28 OCT 09 '90 13:01:26

AREA%

| RT | AREA | TYPE | AR/HT | AREA% |
|---|---|---|---|---|
| 3.148 | 3.4262E+07 | PB | 4.245 | 87.189 |
| 5.221 | 5034300 | BB | 0.305 | 12.811 |

TOTAL AREA= 3.9296E+07
AUL FACTOR= 1.0000E+00



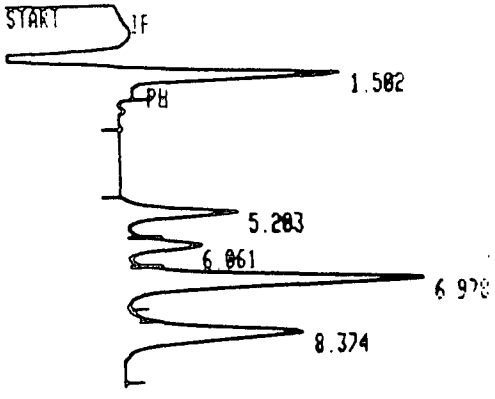

STOP

RUN # 29 OCT 09 '90 13:13:20

AREA%

| RT | AREA | TYPE | AR/HT | AREA% |
|---|---|---|---|---|
| 1.504 | 2.1722E+07 | SBB | 0.395 | 35.492 |
| 5.198 | 5093800 | PB | 0.255 | 8.323 |
| 6.859 | 3745800 | BB | 0.279 | 6.120 |
| 6.963 | 1.6910E+07 | BB | 0.348 | 27.629 |
| 8.341 | 1.3732E+07 | BB | 0.434 | 22.437 |

TOTAL AREA= 6.1204E+07
AUL FACTOR= 1.0000E+00




STOP

## SUMMARY & FUTURE PLANS

BOTH CATION AND ANION ANALYSIS TO BE DONE FOR

A. OVULES AT THE TIME OF FERTILIZATION & EARLY PRO-EMBRYO FORMATION

B. MATURE SEEDS AT THE TIME OF HARVEST.

DATA SO OBTAINED WOULD BE USED FOR COMPARISON WITH ELEMENTS OF SYNTHETIC CULTURE MEDIA.

DATA ON ANION ANALYSIS DID NOT REVEAL PEAKS FOR NITRATE OR NITRITE

TWO PEAKS STILL NEED TO BE IDENTIFIED

ESTIMATION OF TOTAL NITROGEN

# DEVELOPMENT AND MATURATION OF SOMATIC EMBRYOS DOUGLAS-FIR

NAGMANI, R. DEDRA EVANS

## OBJECTIVES

ESTABLISH SUSPENSION CULTURES

PROMOTE DEVELOPMENT & MATURATION OF SOMATIC EMBRYOS IN SUSPENSION CULTURES IN LARGE NUMBERS

TO DEVELOPE A PROTOCOL OPTIMAL FOR ATLEAST 3 EC LINES REPRESENTING 3 GENOTYPES

## EXPERIMENTS

1. TO TEST THE EFFECT OF CAESEIN HYDROLYSATE AS NITROGEN SOURCE ON DEVELOPMENT & MATURATION
2. TO TEST THE EFFECT OF MALTOSE vs SUCROSE
3. TO TEST THE EFFECT OF AUTOCLAVED CHO vs FILTER STERILIZED
4. TO TEST THE EFFECT OF DIFFERENT LEVELS OF ABA

# DEVELOPMENT AND MATURATION OF SOMATIC EMBRYOS OF DOUGLAS-FIR

RP. NO: 11/21/90

RESEARCHERS ORIGINATING STUDY: NAGMANI & DEDRA EVANS

TREATMENTS :

1. 1/2 mMSG + MALTOSE 0.8M ( 3% ) ( AUTOCLAVED )
2. 1/2 mMSG + MALTOSE 0.8M ( 3% ) ( AUTOCLAVED) + 1µM IBA
3. 1/2 mMSG + MALTOSE 0.8M ( 3%) (AUTOCLAVED) + 1µM IBA +10 µM ABA
4. 1/2mMSCG + MALTOSE 0.8 M (3%) (AUTOCLAVED) + 10µM ABA
5. 1/2 mMSCG + MALTOSE 0.8M (3%) (FILTER STERILIZE) +10µM ABA
6. 1/2 mMSCG + SUCROSE 0.8 M (3%) ( AUTOCLAVED) + 10µM ABA
7. 1/2 mMSCG + SUCROSE 0.8 M (3%) (FILTER STERILIZE) + 10µM ABA
8. 1/2 mMSG + MALTOSE 0.8 M ( 3%) ( AUTOCLAVED) + 10µM ABA
9. 1/2 mMSG + MALTOSE 0.8 M ( 3%) (FILTER STERILIZE) + 10 µM ABA
10. 1/2 mMSG + SUCROSE 0.8 M (3% )( AUTOCLAVED) + 10 µM ABA
11. 1/2 mMSG + SUCROSE 0.8 M (3%) ( FILTER STERILIZE) + 10µM ABA

( G= GLN = 500 mg/L; C= CH = 1000 mg/L )

## SUMMARY

PRELIMINARY EXPERIMENTS HAVE INDICATED:

CAESEIN HYDROLYSATE IS NOT NECESSARY FOR DEVELOPMENT & MATURATION ?

MALTOSE ALONE PROMOTES DEVELOPMENT & MATURATION

MALTOSE WITH ABA AT 10uM ENHANCES SYNCHRONOUS DEVELOPMENT OF PRE-COTYLEDONARY EMBRYOS IN LARGE NUMBERS

## FUTURE PLANS

1. EFFECT OF CARBOHYDRATES; GLUCOSE, MALTOSE & SUCROSE AT 1 ½ ; 3, 6, 9 & 12%

2. OPTIMAL SOURCE OF CHO & OPTIMAL LEVEL + ABA ( 0,2,5,10,20,40 & 80 uM )

3. STATISTICAL EVALUATION OF DATA

# STATUS OF HARDWOOD PLANT MATERIAL

SONJA OZTURK

SHANNON JOHNSON

YOLANDA POWELL

RON DINUS

# OBJECTIVES

## BUILD & MAINTAIN "CLEAN" GREENHOUSE POPULATIONS

## ESTABLISH & MAINTAIN STOCK CULTURES

## RESULTS, CONT'D

## COTTONWOOD STOCK CULTURES

| CLONE/CULTURE CODE | CULTURE ORIGIN LEAF | PETIOLE | STEM | SOURCE |
|---|---|---|---|---|
| C175 | + | NA | + | UNIV. OF NB |
| K417 | - | - | + | UNIV. OF KY |
| ST66 | - | - | + | JAMES RIVER |
| ST70 | - | - | + | SAME |
| ST72 | - | - | + | SAME |
| ST75 | - | - | + | SAME |

BEST MEDIA: INITIATION, DKW + THIDIAZURON (0.1 uM)
MAINTENANCE, DKW + NAA (0.1 uM) & BA (1.0 uM)

# RESULTS

## GREENHOUSE POPULATIONS:

BUILT TO 7 COTTONWOOD CLONES

INCLUDES 5 GENETICALLY IMPROVED SOUTHERN CLONES

K417 - UNIV. OF KY

ST 66 - JAMES RIVER CORP.

ST 70 - SAME

ST 72 - SAME

ST 75 - SAME

OTHERS - C175 - UNIV. OF NB

XD-44-65-5-2 - IPST (UNIV. OF MN)

# CONCLUSIONS/PLANS

## CONCLUSIONS:

### GREENHOUSE -

HAVE LARGER ARRAY OF GENOTYPES, 7 TOTAL

INCLUDES 5 GENETICALLY IMPROVED ONES, USEFUL IN SOUTH

### CULTURES -

BUILT CULTURES TO INCLUDE 6 CLONES

ADDED 5 NEW GENETICALLY IMPROVED CLONES

## PLANS:

### GREENHOUSE -

MAINTAIN & FURTHER ENLARGE ARRAY OF GENOTYPES

### CULTURES -

INCREASE CULTURE NUMBERS / CLONE

RAISE NUMBER GENOTYPES IN CULTURE

TEST OSU/UNB INITIATION PROTOCOL

# Promotion of Additional Auxin Biosynthesis in *Populus deltoides* through Genetic Engineering

**D.T. Webb, J.N. Mathis & R. Dinus - Principal Investigators**

**P. Shorter - M. Sc. Student**

# OBJECTIVES

1. Develop tissue culture systems suitable for transformation

2. Use cocultivation with genetically engineered *Agrobacterium* to transfer auxin biosynthesis gene(s)

3. Select and regenerate transformed plants

4. Analyze for phenotypic traits

5. Assay for increased IAA synthesis

6. Confirm presence of foreign gene

7. Use with other poplar clones

8. Use with other genes

# BACKGROUND

1. *Agrobacterium* transfers two genes affecting auxin biosynthesis during transformation

2. These genes (iaaM & iaaH) -> enzymes -> tryptophane -> IAA

   A. Tryptophane -> iaaM -> indole-3-acetamide

   B. Indole-3-acetamide -> iaaH -> indole-3-acetic acid (IAA)

   C. Genes function in bacteria

   D. Genes are not known to be present in normal plants

   E. Activity of iaaM alone should not lead to IAA production

3. Petunia transformed with iaaH

   A. Use indole-3-acetamide as a substrate -> IAA

   B. Have normal phenotype

## BACKGROUND

4. Petunia transformed with iaaM (Constituitive Promoter)
   - A. Have elevated levels of IAA
   - B. Have abnormal phenotype
     1. Increased Apical Dominance
     2. Increased Cell Size
     3. Elongated Internodes
     4. More Woody Stems
     5. More Secondary Xylem & Phloem
     6. Larger & Thicker Leaves
     7. Curled & Narrow Leaves
     8. Adventitous Roots (Leaves & Stems)
     9. Abnormal Roots
     10. Fertile Plants -> Seeds
5. Petunia transformed with seed storage protein promotor
   - A. Normal Phenotype
   - B. Expression in the Seed
   - C. Mendelian Inheritance

# RESULTS

1. **Improved leaf section regeneration system**

2. **Improved internode regeneration system**

3. **Antibiotic screening**

4. **Genes & *Agrobacterium* strains obtained**

## NEXT STEPS/OPTIONS

1. Develop *Agrobacterium* vector for iaaM gene
2. Perform co-cultivation experiment
3. Select & regenerate transformants
4. Analyze putuaive transformants
   - A. Phenotype
   - B. Presence of iaaM gene
   - C. IAA content
5. Identify tissue specific & stage specific promoters
6. Make new gene constructs
7. Transform with new constructs -> desirable traits
8. Extend to other *P. deltoides* clones
9. Use with other genes (herbicide resistance)

# Genetic Transformation of *Populus deltoides* with Economically Important Genes

**J.N. Mathis, D.W. Webb, R. Dinus - Principal Investigators**

**C.J. Stephens - Research Assistant**

**P. Shorter - M.S. Student**

# Methods for Transformation of *Populus deltoides* with Glyphosate Resistance

1. Co-cultivation Agrobacterium and leaf discs (using Monsanto construct)

2. Development of transformants in B5 medium as described by:

   M. De Block (Plant Physiol. 93:1110-1116)

   1. Mes and Ca-gluconate will be used to buffer the media and avoid shoot tip necrosis

   2. Temperature below 25°C

   3. Greenhouse tests of plants will be performed, then field trials

# Development of Somaclonal Variants of *Populus deltoides* with Glyphosate Resistance

J.N. Mathis, R. Dinus - Principal Investigator

C.J. Stephens - Technical Assistant

Step 1

# Development of Growth Curves For Cell Cultures of *P. deltoides* C175

J.N. Mathis, R. Dinus - Principal Investigators

S. Ozturk, S. Johnson - Technical Assistants

M. Naik - Ga. Tech Undergraduate

S. Bergman - Ga. Tech Undergraduate

## Objective

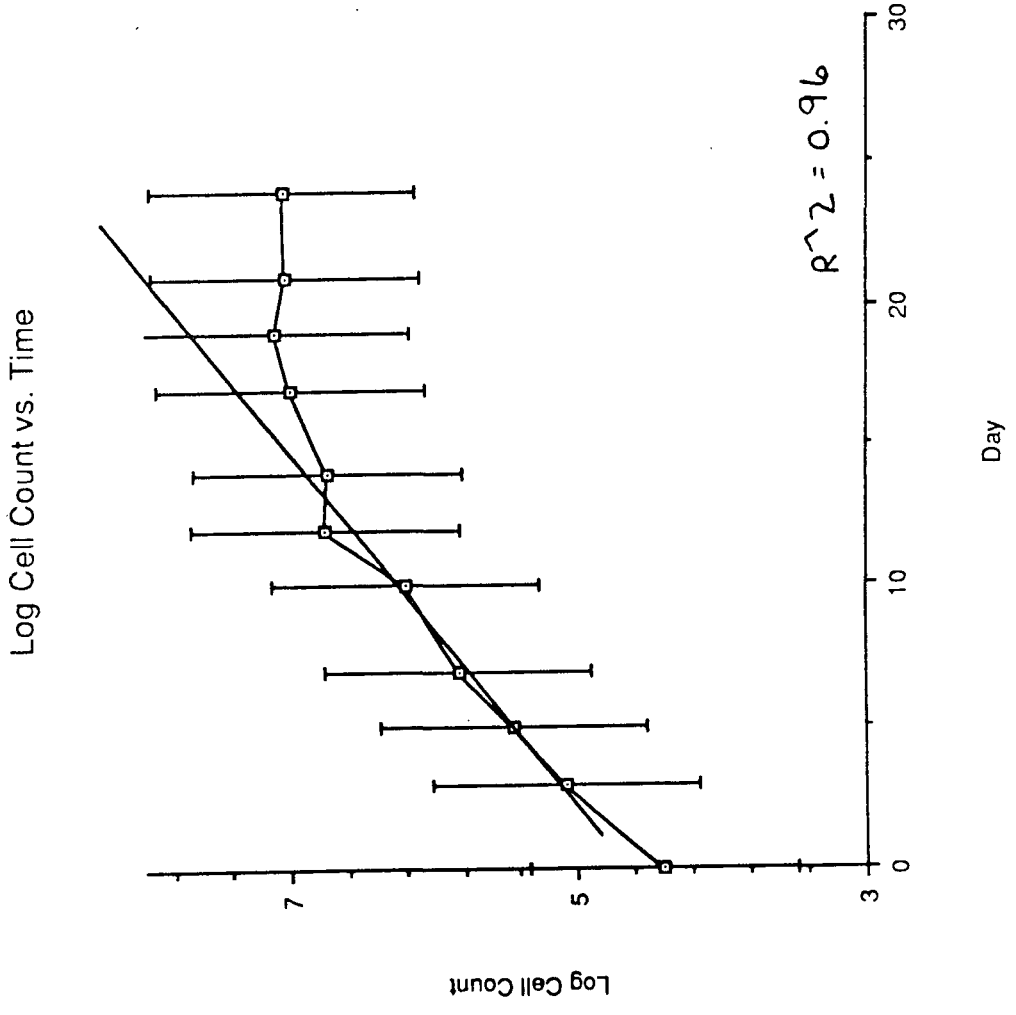
To quantify the growth kinetics of *P. deltoides* C175 in order to assist in:

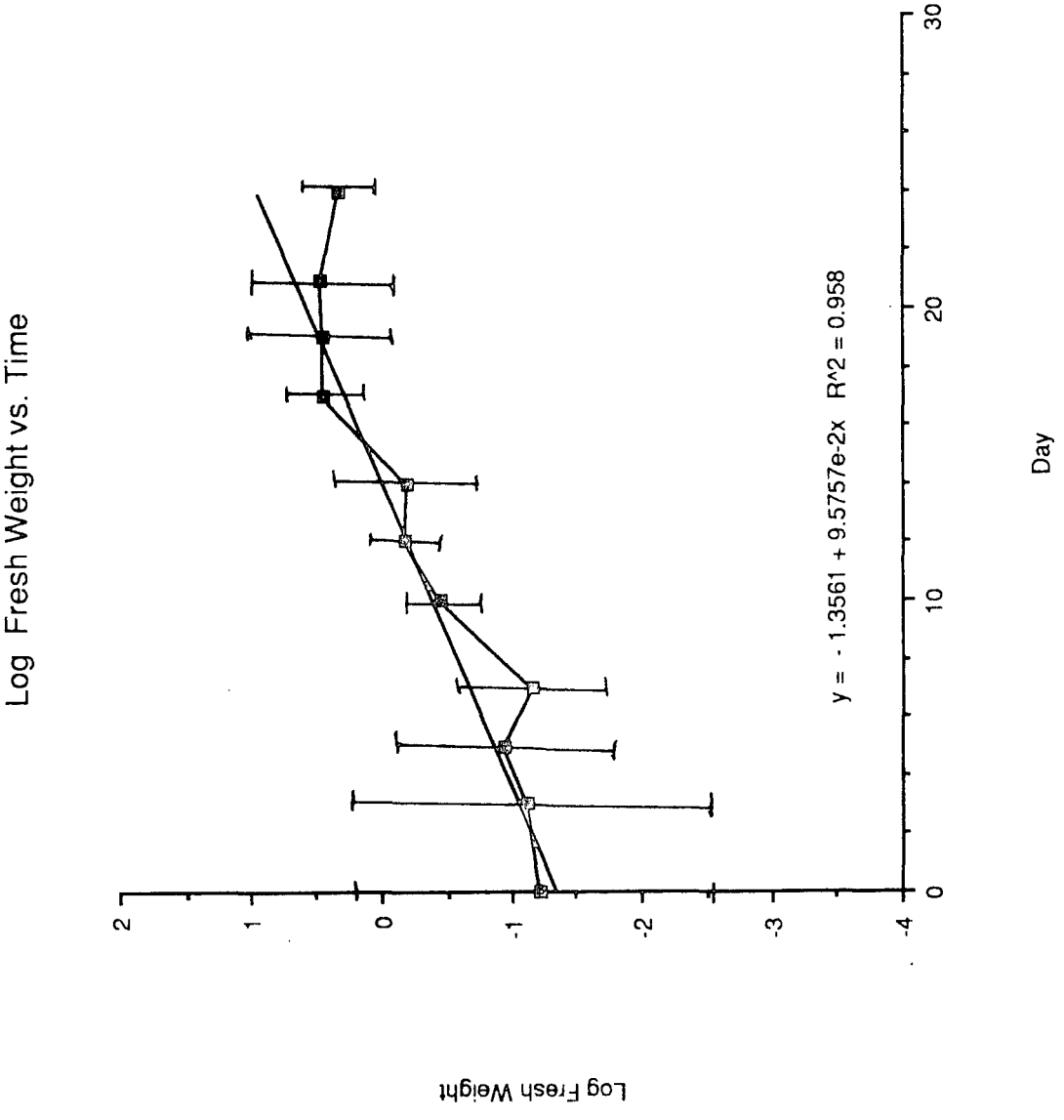
1. Developing somaclonal variants
2. Studying xylogenic cultures
3. Developing transformation systems

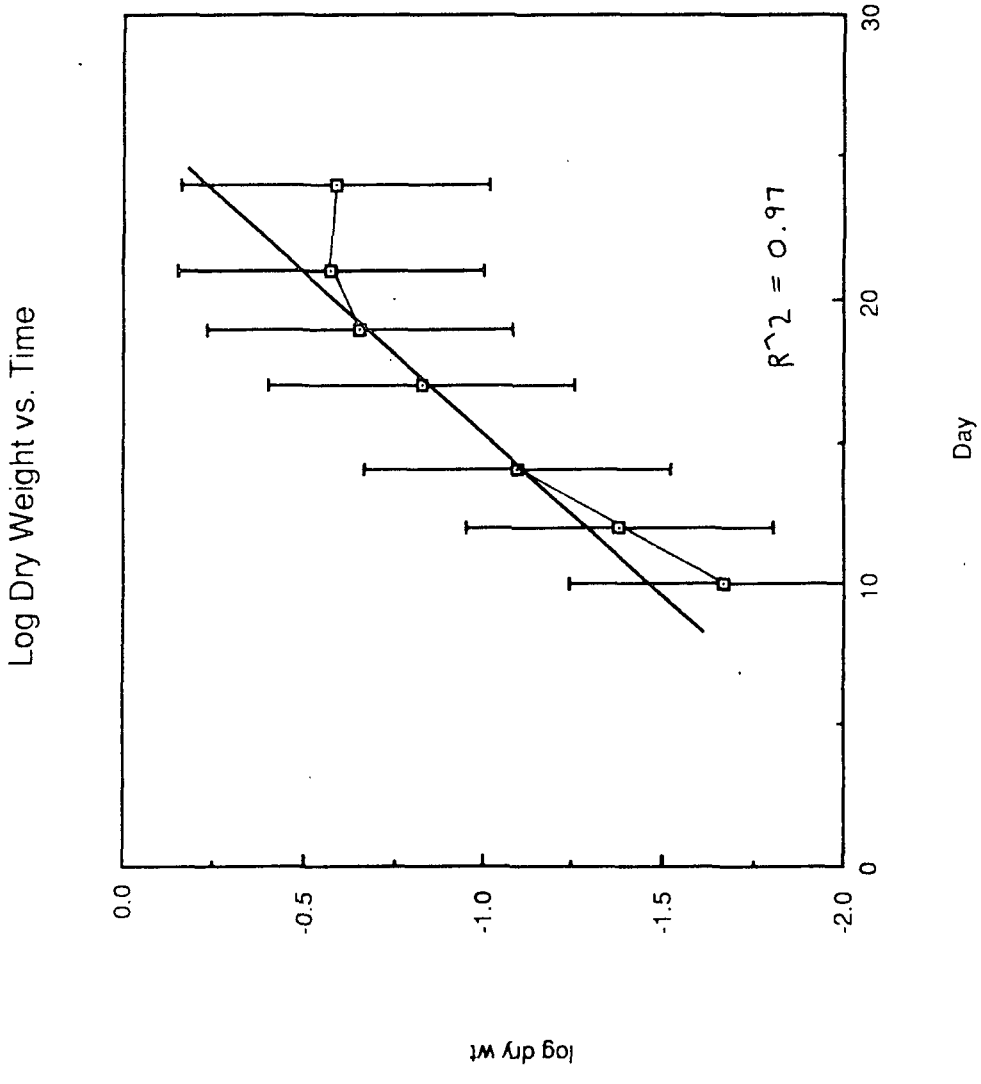
## Experimental Approach

**Measure cell growth in five different ways with five replicate cultures and compare each method**

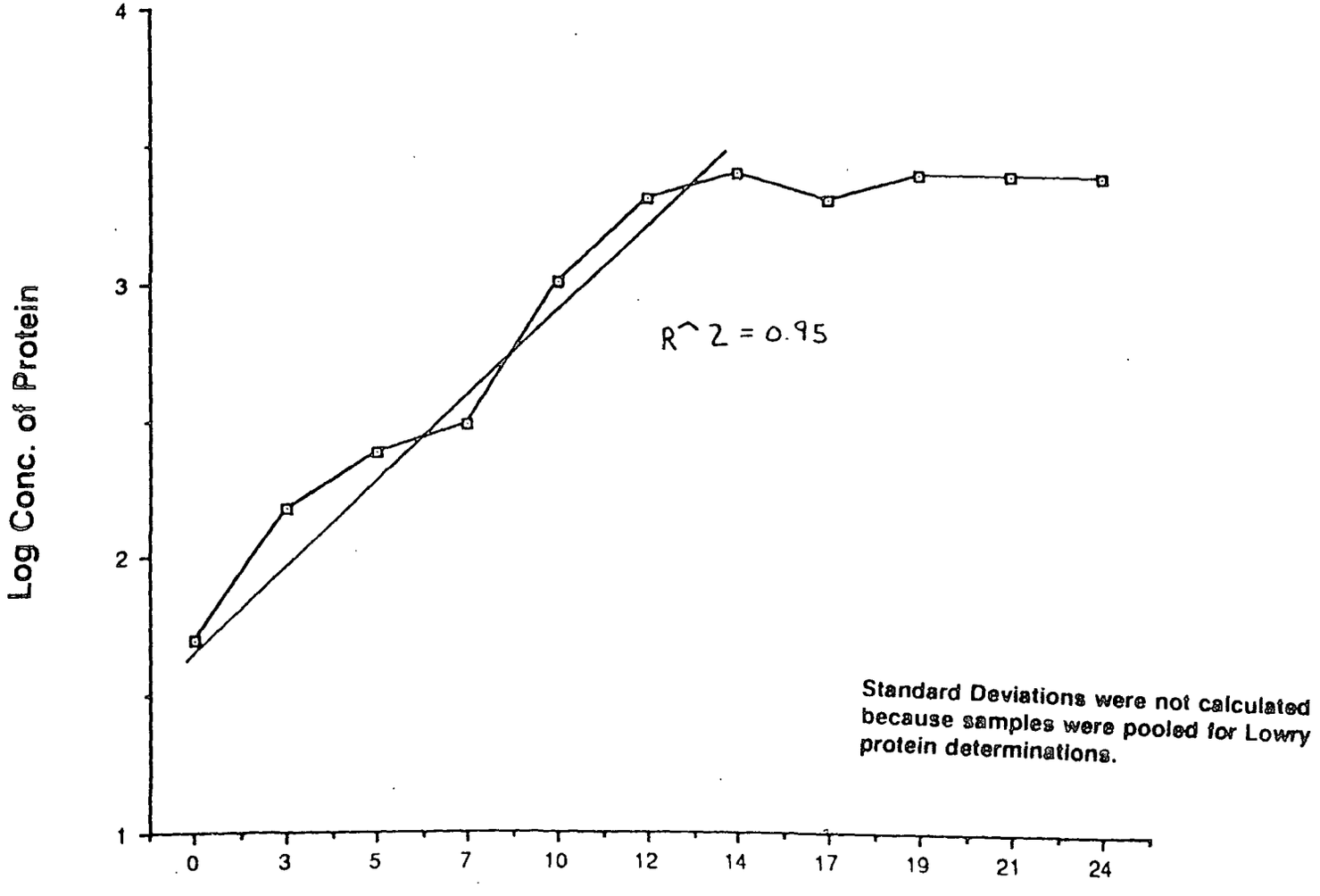
1. **Cell Number**
2. **Fresh Weight**
3. **Dry Weight**
4. **Total Protein by Lowry**
5. **Settled Cell Volume**


Log Cell Count vs. Time
R^2 = 0.96
Log Cell Count
7
5
3
0
10
20
30
Day

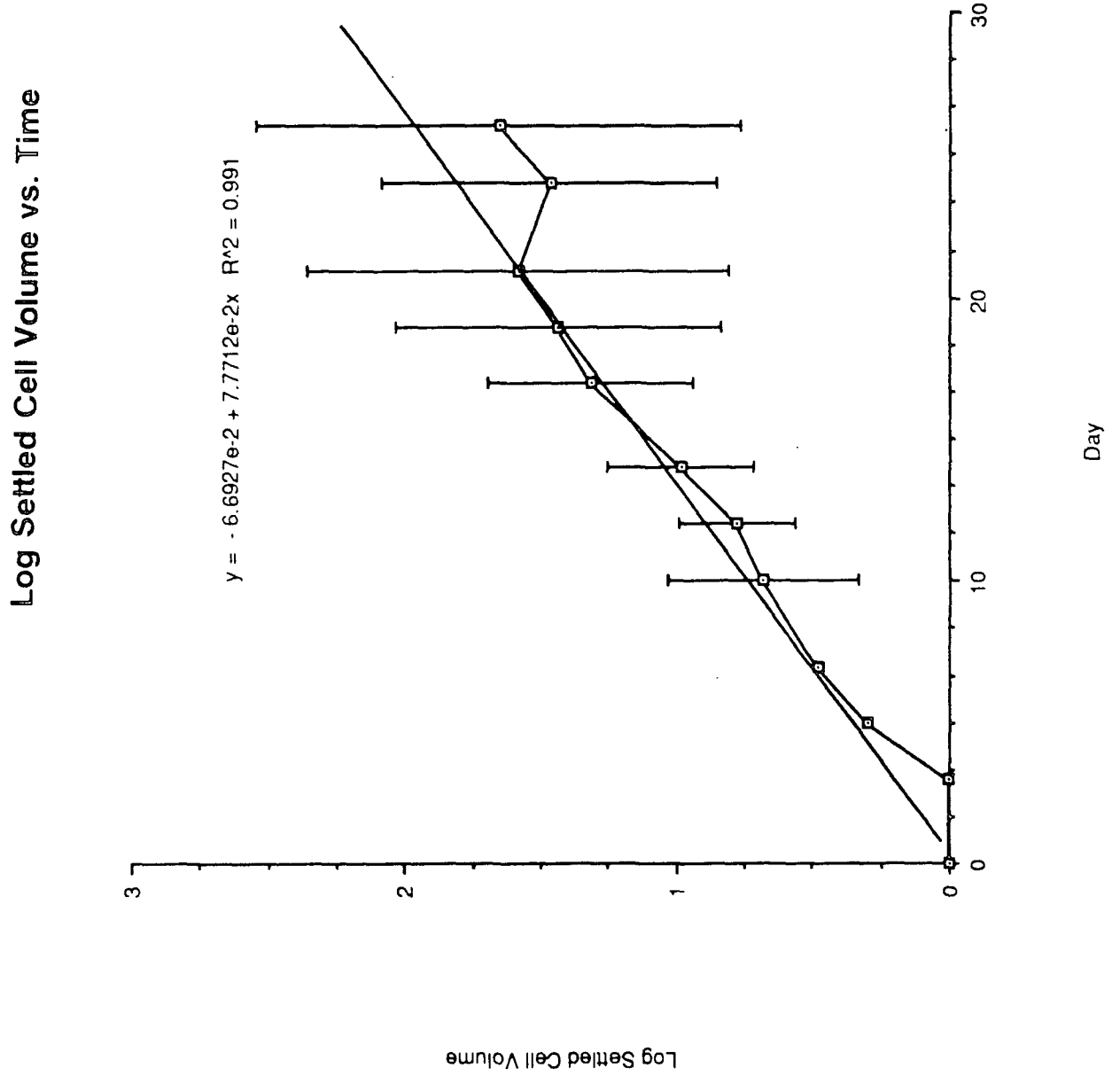

Log Fresh Weight vs. Time
y = - 1.3561 + 9.5757e-2x R^2 = 0.958
Log Fresh Weight
Day
2
1
0
-1
-2
-3
-4
0
10
20
30


Log Dry Weight vs. Time
R^2 = 0.97
log dry wt
0.0
-0.5
-1.0
-1.5
-2.0
0
10
20
30
Day

## Log Concentration of Protein vs. Time




Standard Deviations were not calculated because samples were pooled for Lowry protein determinations.


Log Settled Cell Volume vs. Time
y = - 6.6927e-2 + 7.7712e-2x R^2 = 0.991
Log Settled Cell Volume
Day
0
1
2
3
0
10
20
30

# SUMMARY of RESULTS

| METHODS | MEAN GENERATION TIME IN DAYS |
|---|---|
| Cell Count | 3.0 |
| Fresh Weight | 3.3 |
| Dry Weight | 3.3 |
| Protein Content | 3.0 |
| Settled Cell Volume | 4.5 |

# CONCLUSIONS

*P. deltoides* cultures C175 in MS media with 3% sucrose, 1mg/L 2,4D and 0.1 mg/L BA have a generation time of 3 days and are in exponential growth phase between 5 and 19 days under several specified conditions:

1. only 20% of the culture flask is taken up with medium to allow adequate aeration

2. a 10% inoculum is used to start cultures

3. a growth temperature of 24°C and a shaking speed of 125 rpm

STEP 2

# Development of $LD_{100}$ for Glyphosate

J.N. Mathis, R. Dinus - Principal Investigators

C.J. Stephens - Technical Assistance

## Methods

Plate cells that have been growing in various levels of glyphosate from 0-512 $\mu$M and stain culture with tetrazolium to determine viability.

## Results

### Tetrazolium - 128 $\mu$M initial experiment

### Plating - still underway

## Future Directions to Develop Glyphosate Resistant Somaclonal Variants

1. Plate populations from glyphosate challenge experiment

2. Look for survivors in cultures above or slightly below apparent $LD_{100}$

3. Rechallenge putative glyphosate resistant clones with potentially lethal doses of glyphosate. Keep increasing dosage as done by several other investigators who have developed glyphosate resistant somaclonal variants.

4. Place resistant calli on differentiation media. Develop plants as outlined by R. Uddin

5. Test resistant plants in the greenhouse and field

# INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY

## Atlanta, Georgia

## FOREST BIOLOGY

## PROJECT ADVISORY COMMITTEE

## STUDENT PROJECTS

### Student Research Comprehensive List

**December 18, 1990**

## STUDENT RESEARCH - COMPREHENSIVE LIST

Completed in 1990

Michael Wood - M.Sc., Effect of cold shocking on cell cultures of Larix decidua. Advisor, Dinus.

In Progress

Teri Ard - Special Student, Project pending. Advisor, Dinus.

David Barzyk - M.Sc., Development of a fiber optics system to determine the in vivo pH of developing Pinus taeda seeds. Advisor, Dinus.

James Bond - Ph.D., A Raman microspectroscopic investigation of the patterns of molecular order in secondary walls of southern pine tracheids. Committee participation, Dinus.

James Bradburne - Ph.D. (GT), Molecular characterization of ineffective Bradyrhizobium japonicum USDA 110 variants and differences in signal transduction pathways between effective and ineffective Bradyrhizobium japonicum USDA 110 variants. Advisor, Mathis.

Rebecca Champion - Ph.D. (GT), Strain X cultivar interactions; Effects of nitrogen-fixing and non-nitrogen-fixing Bradyrhizobium japonicum USDA 110 on nodulation and nitrogen fixation. Advisor, Mathis.

Lois Forde - M.Sc., Phenylalanine ammonia lyase and lignin biosynthesis. Advisors, Conners and Dinus.

Robert Golden - M.Sc. (GT), Development of a rapid clinical diagnostic procedure for the most common cystic fibrosis gene. Advisor, Mathis.

Rene Kapik - Ph.D., Recently admitted, starting A390 problems. Advisor, Dinus.

Jim Kramer - M.Sc., Pulping and papermaking properties of Florida-grown Eucalyptus amplifolia. Advisors, Dinus and McDonough.

Tom Ptacek - M.Sc., Variability of wood, fiber, and pulping properties as affected by cloning. Advisor, Dinus.

Peasely Shorter - M.Sc., Promotion of additional auxin synthesis in Populus deltoides via transformation with Agrobacterium tumefaciens. Advisor, Webb.

Colleen Walker - Ph.D., Development of a biomimetic approach for pulp bleaching. Advisor, Dinus.

Michael Wood - Ph.D., Completing A390 problems; Decision on dissertation topic pending. Advisor, Dinus.

FORWARD PLANNING

# SUMMARY, PLANS, & NEEDS

Ron Dinus/Brian Stanton

# KEY ISSUES

QUESTION: WHY BE CONCERNED?

KEY GOALS, SET IN FALL, 1989

MANY NEW DEVELOPMENTS

KEEP, DROP, OR ADJUST?

ORIGINAL PURPOSE

PERIODIC, OBJECTIVE EVALUATION EVERY 2 OR 3 YRS

DECIDE TO CONTINUE, TURN, OR STOP

IN ADDITION TO BUT COINCIDENT WITH

IPST STRATEGIC PLAN

NEAR-TERM PROJECT PLANS

BASES FOR EVALUATION

PROCESS STEP IMPROVEMENTS

ENHANCED UNDERSTANDING & NEW KNOWLEDGE

NEW OPPORTUNITIES

# "SOMATIC SEEDLING" YIELDS, ADVANCED SPRUCE SYSTEMS

## ORGANIZATION: BC RESEARCH CENTRE

## SPECIES: INTERIOR SPRUCE

| # GENOTYPES (MOTHER TREES) | SEEDLINGS (#) | NURSERY SURVIVAL (%) |
|---|---|---|
| 71 (6) | 1200 | 80 |

**Authors Conclusion: Can be used to produce planting stock for reforestation from an array of genotypes.**

## ***** KEY GOALS *****

1) **INCREASE DEVELOPMENT/MATURATION FREQUENCY IN MODEL SPECIES TO 25% ON A REPEATABLE BASIS, AND EXTEND TO TARGET SPECIES.**

**IE., MOVE 250 OR MORE OF THE 500 - 1000 POTENTIAL EMBRYOS PRESENT PER GRAM OF EMBRYOGENIC CALLUS TO MATURITY.**

**REQUIRES IMPROVED UNDERSTANDING OF ZYGOTIC SYSTEM.**

2) **IMPROVE CONVERSION PROTOCOLS FOR MODEL SPECIES & PRODUCE A POPULATION OF SUITABLE SIZE & VARIABILITY FOR REPLICATED GREENHOUSE & FIELD TRIALS.**

**IE., ACCUMULATE 375 USABLE SEEDLINGS FROM EACH OF ONE OR MORE CALLUS LINES REPRESENTING EACH OF SEVERAL DONOR TREES.**

**REQUIRES IMPROVED UNDERSTANDING OF ZYGOTIC SYSTEM, REFINED GERMINATION METHODS, & IMPROVED ACCLIMATIZATION PROCEDURES.**

## ***** KEY GOALS *****

3) RAISE INITIATION FREQUENCIES IN TARGET SPECIES TO 10% ON A REPEATABLE BASIS ACROSS EXPERIMENTS, SEASONS, AND GENOTYPES.

MAIN INTENT: IMPROVE RELIABILITY OF PROCESS STEP, AND GAIN ABILITY TO OBTAIN EMBRYOGENIC CALLUS AT WILL & WITH EASE FOR RESEARCH ON OTHER CRITICAL STEPS.

HONORABLE MENTION: OBTAIN EMBRYOGENIC CALLUS OF AT LEAST ONE SOFTWOOD, FROM EXPLANTS OF TREES OLD ENOUGH TO HAVE BEEN PROVEN GENETICALLY SUPERIOR.

# KEY ISSUES - STAFF VIEWS & SUGGESTIONS

## NORWAY SPRUCE - NEED STRATEGIC DECISION

STOP ALL WORK EXCEPT -

- SOMATIC & ZYGOTIC COMPARISONS
- CONVERSION PROTOCOLS
- FIDELITY
- INITIATION FROM OLDER DONORS

## LOBLOLLY PINE - INCREASE EFFORT

INITIATION - RETAIN FORMER KEY GOAL,

10% ACROSS TESTS, SEASONS, & GENOTYPES

MATURATION - COMBINE & PUSH OLD + NEW THRUSTS

- IN VULUO ENVIRONMENT
- CHO & ABA EFFECTS
- MOLECULAR BASIS OF CHO EFFECT
- LIQUID VS SOLID MEDIA
- SYNCHRONY, COUNTING, & HARVESTING

CONVERSION PROTOCOLS - ADAPT FROM NORWAY SPRUCE

FIDELITY - ADAPT FROM NORWAY SPRUCE

**DOUGLAS-FIR**

**INITIATION** - DEFER, UNLESS NEED NEW LINES

**MATURATION** - CONTINUE AT PRESENT LEVEL

CHO & ABA EFFECTS
LIQUID VS SOLID MEDIA
SYNCHRONY, COUNTING, & HARVESTING

LEARN & GENERALIZE TO LOBLOLLY

**CONVERSION & FIDELITY**

ADAPT FROM NORWAY SPRUCE
EXTEND TO LOBLOLLY

## KEY ISSUES - REQUEST FOR ASSISTANCE

**APPOINT SPECIAL SUBCOMMITTEE(S)**

**CONSULT OUTSIDE EXPERTS**

**BUILD RECOMMENDED LIST & EXPLANATION**

**CIRCULATE TO ALL MEMBERS**

**DISCUSS & APPROVE AT APRIL MEETING**

# NEW DIRECTIONS

**EXTERNAL FUNDING ???????**

**EARLIER & OVER THE HILL:**

**USDA - LIGNIN BIOSYNTHESIS, 01/90**
**RD/DW + UGA - NOT FUNDED**

**NSF - SEED COMPOSITION, 01/90**
**NR/SB - NOT FUNDED**

**USDA - LIGNIN DISTRIBUTION & STRUCTURE, 03/90**
**RD/DW + UGA - NOT FUNDED, NEAR-MISS**

**NSF - NOVEL ENZYMES IN LIGNIFICATION, 01/90**
**UGA + RD - WITHDRAWN, PI LEFT UGA**

**SUBMITTED & WAITING:**

**NSF - LIGNIFICATION, 06/90**
**UGA/RD/DW - NO WORD YET**

**DOE - SAME**

**RECENTLY CONTRIVED & SUBMITTED:**

**MEMBER COMPANY - IDENTIFYING & PROTECTING**
**ELITE GERMPLASM, 10/90 - JM**

**NSF - LIGNIN BIOSYNTHESIS, 12/90**
**UGA/DW**

**NSF - LIGNIN DISTRIBUTION & STRUCTURE, 12/90**
**UGA/RD**

## NEW DIRECTIONS, CONT'D

NEAR-NEAR-TERM PLANS:

SOFTWOODS:

COMPLETE SUMMER INITIATION EXPERIMENTS (LP)

ESTABLISH WINTER INITIATION TRIALS (LP)

COMPLETE PROJECTED MATURATION TESTS (LP & DF)

INITIATE WORK ON MOLECULAR BASIS
OF CHO EFFECTS ON MATURATION (LP)

COMPARE SOMATIC/ZYGOTIC DEVELOPMENT (NS)

FACILITATE STUDENT PROJECTS

RECOMMEND NEW/REVISED KEY GOALS, STAFF/PAC

## NEW DIRECTIONS, CONT'D

### NEAR-NEAR-TERM PLANS:

#### BIOCHEMISTRY:

COMPLETE RECRUITING/HIRING

ACQUIRE LAST SUPPLIES/EQUIPMENT

SUPPORT SOMATIC/ZYGOTIC COMPARISONS

ADAPT/REFINE METHODS FOR WORK ON MOLECULAR

BASIS OF CHO EFFECTS IN MATURATION

REFINE METHODS FOR GENE TRANSFER VIA At

FACILITATE STUDENT PROJECT ON GENE TRANSFER

DEVELOP/ADAPT METHODS FOR ASSESSING FIDELITY

## NEW DIRECTIONS, CONT'D

NEAR-NEAR-TERM PLANS:

HARDWOODS:

REPLACE POST-DOCTORAL FELLOW, IF APPROVED

ENLARGE GREENHOUSE & STOCK CULTURE POPULATIONS

COMPLETE FIRST WORK ON SOMACLONAL VARIATION/SELECTION

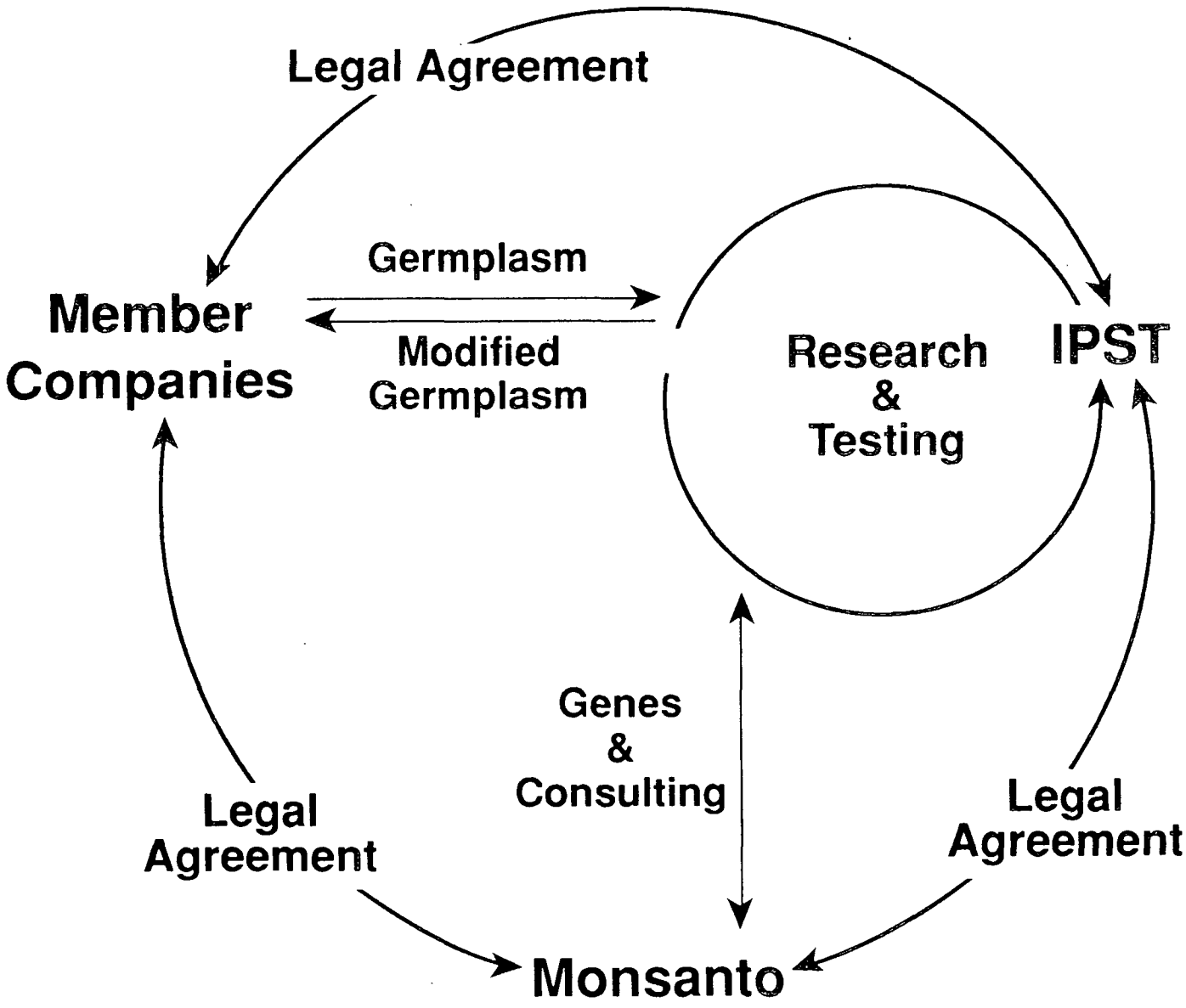
EXTEND LEAF SECTION SYSTEM TO MORE GENOTYPES

FINALIZE AGREEMENT WITH MONSANTO;
SECURE GENE FOR GLYPHOSATE TOLERANCE

START WORK ON TRANSFER OF GLYPHOSATE TOLERANCE GENE

FACILITATE STUDENT PROJECT ON LIGNIFICATION

# Herbicide Tolerance Gene: Status & Process



## FORWARD PLANNING, COMMITTEE ISSUES

TERMS OF OFFICE

OFFICERS

ALTERNATES/SUBSTITUTES

EXTERNAL SPECIALISTS

MINUTES

FUTURE MEETINGS

DEFINITE - APRIL 1, 1990 (FORMAL)

OTHERS - NEEDED; WHEN & WHERE?

HAPPY HOLIDAYS!!!!